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## **Somatic RET mutation in a patient with pigmented adrenal pheochromocytoma**

Maison, Nicole ; Korpershoek, Esther ; Eisenhofer, Graeme ; Robledo, Mercedes ; de Krijger, Ronald ; Beuschlein, Felix

**Abstract:** UNLABELLED Pheochromocytomas (PCC) and paraganglioma (PGL) are rare neuroendocrine tumors arising from chromaffin cells of the neural crest. Mutations in the RET-proto-oncogene are associated with sporadic pheochromocytoma, familial or sporadic medullary thyroid carcinoma (MTC) and multiple endocrine neoplasia type 2. In the past, only few cases of pigmented PCCs, PGLs, and one case of pigmented MTC have been reported in the literature. Herein, we present the case of a 77-year old woman with a history of Tako-tsubo-cardiomyopathy and laboratory, as well as radiological, high suspicion of pheochromocytoma, who underwent left-sided adrenalectomy. The 3 cm tumor, which was located on the upper pole of the left adrenal, appeared highly pigmented with dark red to black color. Histologic examinations revealed highly pleomorphic cells with bizarre, huge hyperchromatic nuclei, that immunohistochemically were positive for chromogranin A and synaptophysin, focally positive for HMB45 and negative for melan A. These clinical and pathological features led to the diagnosis of the rare variant of a melanotic 'black' pheochromocytoma. In our case a somatic RET mutation in exon 16 (RET c.2753T>C, p.Met918Thy) was detected by targeted next generation sequencing. In summary, this case represents a rare variant of catecholamine-producing tumor with distinct histological features. A potential relationship between the phenotype, the cellular origin and the genetic alterations is discussed. **LEARNING POINTS** Pheochromocytoma is a rare neuroendocrine tumor. Pigmentation is seen in several types of tumors arising from the neural crest. The macroscopic black aspect can mislead to the diagnosis of a metastasis deriving from a malignant melanoma. RET mutation are seen in catecholamine and non-catecholamine producing tumors of the same cellular origin.

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# Somatic *RET* mutation in a patient with pigmented adrenal pheochromocytoma

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## Summary

Pheochromocytomas (PCC) and paraganglioma (PGL) are rare neuroendocrine tumors arising from chromaffin cells of the neural crest. Mutations in the *RET*-proto-oncogene are associated with sporadic pheochromocytoma, familial or sporadic medullary thyroid carcinoma (MTC) and multiple endocrine neoplasia type 2. In the past, only few cases of pigmented PCCs, PGLs, and one case of pigmented MTC have been reported in the literature. Herein, we present the case of a 77-year old woman with a history of Tako-tsubo-cardiomyopathy and laboratory, as well as radiological, high suspicion of pheochromocytoma, who underwent left-sided adrenalectomy. The 3 cm tumor, which was located on the upper pole of the left adrenal, appeared highly pigmented with dark red to black color. Histologic examinations revealed highly pleomorphic cells with bizarre, huge hyperchromatic nuclei, that immunohistochemically were positive for chromogranin A and synaptophysin, focally positive for HMB45 and negative for melan A. These clinical and pathological features led to the diagnosis of the rare variant of a melanotic 'black' pheochromocytoma. In our case a somatic *RET* mutation in exon 16 (*RET*c.2753T>C, p.Met918Thy) was detected by targeted next generation sequencing. In summary, this case represents a rare variant of catecholamine-producing tumor with distinct histological features. A potential relationship between the phenotype, the cellular origin and the genetic alterations is discussed.

## Learning points:

- Pheochromocytoma is a rare neuroendocrine tumor.
- Pigmentation is seen in several types of tumors arising from the neural crest. The macroscopic black aspect can mislead to the diagnosis of a metastasis deriving from a malignant melanoma.
- *RET* mutation are seen in catecholamine and non-catecholamine producing tumors of the same cellular origin.

## Background

This case represents a rare variant of catecholamine-producing tumor with distinct histological features. A potential relationship between the phenotype, the cellular origin and the genetic alterations is discussed.

## Case presentation

A 77-year-old patient with a history of arterial hypertension, intermittent atrial fibrillation and an episode of Takot-tsubo-cardiomyopathy presented for pre-surgical evaluation of suspected catecholamine excess.



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## Investigation

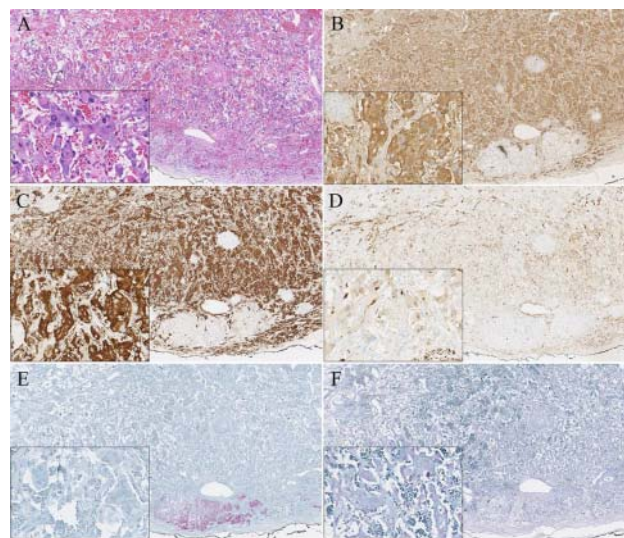
Repeated laboratory examinations documented elevated plasma and urine metanephrine (urine metanephrine 6205.88  $\mu\text{g}/24\text{ h}$  ( $<699.66\text{ }\mu\text{g}/24\text{ h}$ ), normetanephrine 4013.1  $\mu\text{g}/24\text{ h}$  ( $<1698.06\text{ }\mu\text{g}/24\text{ h}$ ) and plasma metanephrine 1460.16  $\text{pmol/l}$  ( $<465.3\text{ pmol/l}$ ), normetanephrine 1116.72  $\text{pmol/l}$  ( $<982.8\text{ pmol/l}$ ). A combined  $^{18}\text{F}$ -DOPA-PET/CT scan revealed presence of a clearly DOPA-positive lesion considered to be arising from the left adrenal gland, measuring  $3.0\times 3.3\text{ cm}$  (Fig. 1).

## Pathologic findings

The resected tumor measured  $3.5\times 3.2\times 2.2\text{ cm}$  and weighed 37g including adjacent adrenal tissue. The tumor was relatively well demarcated with macroscopically red to black color, surrounded by a thin golden-yellow rim representing the preexistent adrenal cortex. Histological slides showed compact groups of cells, partly with huge bizarre, hyperchromatic cell nuclei, and pigment inclusions within the granular cytoplasm. Because of the macroscopical appearance there was initial suspicion of malignant melanoma but in consideration of the clinically and radiologically clear indication of pheochromocytoma, additional immunohistochemical analyses were performed. Tumor cells showed an intense expression of chromogranin A and synaptophysin, S100 was positive only in sustentacular cells (Fig. 2A, B, C and D). HMB45, a marker of melanosomes showed focal



**Figure 1**  
Combined DOPA-PET/CT scan of the abdomen showing an intensively DOPA-positive lesion on the upper left kidney pole.



**Figure 2**

The hematoxylin eosin staining (A) shows highly vascularized tumor tissue, composed of highly pleomorphic, basophilic tumor cells. The positive staining of chromogranin A (B) and synaptophysin (C) of the tumor cells and the presence of S100 positive cells (D) surrounding the tumor cells, confirms this tumor is a pheochromocytoma. Melan-A staining is negative in the tumor cells (E), while the remaining cortex stains positive. HMB45, a marker for melanosomes, shows focal positive cytoplasmic staining (F).

positive cytoplasmic staining (Fig. 2F) and Melan A was negative (Fig. 2E). These features led to the final diagnosis of a melanotic pheochromocytoma (Fig. 3).

Targeted next generation sequencing including *EPAS1*, *FH*, *HRAS*, *KIF1B*, *MAX*, *MDH2*, *MEN1*, *NF1*, *RET*, *SDHA*, *SDHAF1*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *TMEM127* and *VHL* was done on the tumor DNA, which revealed a somatic *RET* mutation in exon 16 (*RET* c.2753T>C, p.Met918Thy), further validated by Sanger sequencing. An underlying MEN2 syndrome was excluded by sequencing the germline DNA.

## Treatment

The patient was treated preoperatively with alpha blockade and underwent uneventful left-sided laparoscopic adrenalectomy. After a short recovery period the patient was discharged from the hospital without need for antihypertensive medication.

## Outcome and follow-up

Follow-up examinations 3 and 6 month after surgery revealed normalization of plasma catecholamines.



**Figure 3**  
Macroscopic aspect of the resected tumor (following paraffin embedding).

Following current guideline suggestions yearly follow-up examinations were recommended (1).

The patient was enrolled into the registry and biobank of the European Network for the Study of Adrenal Tumors ([www.ensat.org](http://www.ensat.org)) upon written informed consent.

## Discussion

Similar to other reports in the literature the black pigmentation of the tumor first led to the suspicion of a metastasis deriving from a malignant melanoma. In fact, black pheochromocytomas (PCC) are very rarely found. In a well defined sub-cohort of 766 PCC/paraganglioma (PGL) patients registered in the ENSAT registry (from Munich, Rotterdam, Madrid, Dresden and Paris) this is the first reported case of a 'black' pheochromocytoma. In the literature, only 18 cases of pigmented PGL and one macro- and 11 microscopically pigmented pheochromocytomas have been reported (Table 1), which emphasizes the rareness of this finding. However, several cases of pigmented tumors, (2), (3) among which neuroblastomas, schwannomas or adrenocortical adenoma, had been described in the past. Ikeda *et al.* (4) reported a case of an medullary thyroid carcinoma (MTC) with a PGL-like pattern and melatonin production. The tumor showed the PGL-typical 'sustentacular cells' as well as pigmented dendritic cells. Genetic analysis unfortunately is not available in this case.

Reported histopathological results regarding the pigment differed, but in most cases lipofuscin, true melanin and neuromelanin were found (5). In our case, the positive

**Table 1** Previous case reports of pigmented pheochromocytoma and pigmented paraganglioma.

Reference	Case No.	Macroscopic pigment	Microscopic pigment	Location	Mutation
<b>Pigmented pheochromocytoma</b>					
Landas <i>et al.</i> (7)	6		6		
Chetty <i>et al.</i> (8)	3		3		
Langer <i>et al.</i> (9)	1		1		
Bellezza <i>et al.</i> (10)	1	1			
Handa <i>et al.</i> (11)	1		1		
Present case	1	1			
<b>Pigmented paraganglioma</b>					
Stout (1935)	1			Ganglion nodosum	NR
Tavassoli (12)	2			Uterus	NR
Paulus <i>et al.</i> (13)	1			Orbit	NR
Hofmann <i>et al.</i> (14)	1			Posterior mediastinum	NR
Küchemann (15)	1			Retroperitoneum	NR
Moran <i>et al.</i> (16)	5			Bladder, spine (2), anterior mediastinum, retroperitoneum	NR
Lack <i>et al.</i> (5)	1			Retroperitoneum	NR
Mikolaenko <i>et al.</i> (17)	1			Heart	NR
Dundr <i>et al.</i> (18)	1			Urinary bladder	NR
Reddy <i>et al.</i> (19)	1			Vagal trunk	NR
Miraldi <i>et al.</i> (20)	1			Heart	NR
Gonnella <i>et al.</i> (21)	1			Heart	NR
Petramala <i>et al.</i> (22)	1			Heart	SDHB
Zhao <i>et al.</i> (23)	1			Kidney	NR

NR, not reported.



HMB45 staining indicates that the pigment results from the presence of melanosomes in at least some of the tumor cells. While true melanin is a pigment produced by melanocytes from L-DOPA, neuromelanin and lipofuscin are byproducts of the macro-autophagy pathway and are associated with breakdown of mitochondria (lipofuscin) and metabolism of catecholamines (neuromelanin) (5). Neuromelanin can also be found in neurons of the substantia nigra. Sulzer *et al.* (6) have shown that L-DOPA induced elevation of cytosolic dopamine is responsible for extended neuromelanin biosynthesis. Thus, neuromelanin and lipofuscin are probably related to excessive catecholamine oxidation. It is suggestive that the tumor cells in pheochromocytoma would contain neuromelanin rather than melanin, also because they possess the enzymes (L-DOPA) to produce it. However, a clear distinction between melanin and neuromelanin cannot be drawn by immunohistochemical staining procedures alone.

The somatic *RET* mutation found in our case is of interest as also non-catecholamine producing but MEN2 associated tumors such as MTC have been reported to occur as hyperpigmented. The rareness of this association clearly indicates that *RET* mutations are not sufficient to cause a hyperpigmentation phenotype. However, it is of interest, that melanocytes, C-cells and adrenal medullary cells share common embryogenetic origins from the neural crest. This common ancestry could be the basis for shared morphological features during tumor development. However, as pigmentation is not an usual feature in non-melanotic tumors, other molecular factors seem to be necessary to induce this particular phenotype.

## Summary

A 'black,' hyperpigmented, adrenal pheochromocytoma is a very rare variant of a catecholamine producing tumor which in the presented case was associated with the presence of melanosomes in tumor cells and the presence of somatic *RET* mutation. As pigmentation has also been described in non-catecholamine-producing tumor cells originating from the neural crest, such as MTC, common mechanisms for pigmentation in these tumor entities could be considered.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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## Patient consent

We confirm that written informed consent has been obtained from the patient.

## Author contribution statement

N Maison was the patient's physician and was involved in paper writing; E Korpershoek conducted the pathology review; G Eisenhofer was responsible for catecholamine determinations; M Robledo conducted genetic studies; R de Krijger conducted the pathology review; F Beuschlein was the patient's physician, and was responsible for the study supervision and paper writing.

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